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Attached please find the following document prepared under this work assignment:

Oil Spill Response Support – Literature Review

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ANAEROBIC DEGRADATION OF PETROLEUM HYDROCARBONS LITERATURE REVIEW

INTRODUCTION

Petroleum hydrocarbons are generally divided into four chemical classes: (1) saturated or aliphatic, (2) aromatic, (3) asphaltenes (phenols, fatty acids, ketones, esters and porphyrins), and (4) resins (pyridines, quinolines, carbazoles, sulfoxides and amides) (44). Petroleum hydrocarbons vary widely in their chemical composition depending upon the source, the degree of refinement (crude oil versus refined oil) and environmental exposure (22). In general, saturated and aromatic hydrocarbons comprise about 80% of crude oil components by mass (71). Once released into the environment, the fate of individual hydrocarbons varies widely due to chemical and physical processes such as volatilization, sorption and dissolution.

The susceptibility of these chemical classes to microbial degradation also varies widely. One of the main factors affecting biodegradability is the chemical structure although other influences such as physical state and toxicity can also affect biodegradability. Some of the short chain *n*-alkanes (C_5-C_{10} homologues) can act as solvents and inhibit microbial growth (6).

Microbial degradation of petroleum hydrocarbons was long thought to occur only in aerobic environments. It was believed that hydrocarbon-degrading microorganisms required molecular oxygen as co-substrate to enzymatically activate hydrocarbons for biodegradation to occur. These activation steps are mediated by mono- or dioxygenases. In addition to being used as an activating agent, oxygen was utilized by aerobic microorganisms as a terminal electron acceptor for respiratory energy conservation for growth (12, 71). Due to the relative inertness of petroleum hydrocarbons, it was believed that a strong oxidant such as oxygen was required for biodegradation to occur and thus hydrocarbons were not degradable in anoxic environments.

Since the late 1980s, an increasing number of research groups have identified novel microorganisms that are capable of metabolizing saturated and aromatic hydrocarbons in anoxic environments. The mechanisms of anaerobic hydrocarbon activation differ from oxygenase attack of hydrocarbons in aerobic environments because there is no equivalent activating agent like oxygen in anoxic environments. These microorganisms utilize nitrate, sulfate or iron(III) as terminal electron acceptors for anaerobic respiration of hydrocarbons. Besides nitrate, sulfate and iron(III) reducing microorganisms, there are microbes that grow in syntrophic co-cultures with other anaerobes and anoxygenic photosynthetic bacteria that utilize petroleum hydrocarbons (71).

During the degradation of petroleum hydrocarbons to metabolic intermediates, reducing equivalents must be transferred to an electron acceptor with a more positive redox potential to facilitate energy conservation for growth. In anoxic environments, energy conservation may be accomplished utilizing nitrate, sulfate and iron(III) as electron acceptors. There are also special microbial consortia which are able to convert reducing equivalents produced from hydrocarbon oxidation to hydrogen gas. The hydrogen gas is utilized by another group of microorganisms (methanogens) in syntrophic relationship with hydrocarbon degraders to make the overall hydrocarbon oxidation reactions thermodynamically feasible. The anoxygenic photosynthetic bacteria have been shown to utilize polar aromatic compounds but no hydrocarbon-utilizing bacteria have been reported (37).

This literature review will summarize the current progress in anaerobic petroleum hydrocarbon degradation and will focus on aliphatic (alkanes and alicyclic compounds) and aromatic hydrocarbons such as BTEX (benzene, toluene, ethylbenzene and xylenes) and polycyclic aromatic hydrocarbons (PAHs).

A number of review articles were used as sources in this literature summary. These articles included reviews by Widdel et al (70), Meckenstock et al (49), Coates et al (17), Boll et al (11), Widdel and Rabus (71), Spormann and Widdel (67), Harwood et al (36), Heider et al (37), Lovley (45) and Krumholz et al (43).

ANAEROBIC DEGRADATION OF SATURATED HYDROCARBONS

Alkanes

The anaerobic oxidation of alkanes, coupled to the reduction of nitrate or sulfate, was first demonstrated in the early 1990s and later confirmed by a number of research groups using pure and enrichment cultures (1, 27, 37, 42, 63, 66). The microbial strain, Hxd3, was shown to completely oxidize hexadecane and other long chain alkanes to carbon dioxide with concomitant reduction of sulfate (1).

Kropp and Suflita showed that sulfate-reducing microcosms were able to completely degrade dodecane under strict anaerobic conditions (42). Experiments showed that the hydrocarbon was activated by addition to fumarate yielding a methyl alkyl succinate metabolite. The reaction involved the addition at a subterminal carbon of the hydrocarbon across the double bond of fumarate. Rabus et al showed that hexane was converted to (1-methylpentyl) succinate (MPS) by the denitrifying bacterium HxN1 (56). In later studies, Wilkes et al proposed that MPS was enzymatically converted to the coenzyme A (CoA) thioester, rearranged to (2-methyl-hexyl)malonyl-CoA, decarboxylated to 4-methyloctonyl CoA and further degraded by β-oxidation to acetyl CoA and propionyl CoA. Fumarate would be regenerated from propionyl-CoA to yield a cyclic process (72). So and Young isolated a sulfate-reducing bacterium, strain AK-1, that was able to anaerobically degrade alkanes (66). The strain produced radiolabeled carbon dioxide from [1-^{14}C]hexadecane with concomitant reduction of sulfate. The stoichiometic molar ratio of sulfate reduction to hexadecane degradation was 89% of the predicted ratio when cell mass production was considered. These findings were confirmed by Callaghan et al in growth experiments comparing strain AK-01, Hxd3 and a sulfate-reducing consortium with hexadecane as substrate. Results showed that strain AK-1 activated hexadecane by a fumarate addition mechanism. A metabolic pathway for degrading hexadecane by strain AK-1 was proposed based on metabolite analysis and identification (15). So and Young provided evidence that alkanes were converted to fatty acids in strain Hxd3 by subterminal carboxylation and removal of two adjacent terminal carbon atoms (15, 65).

In anoxic environments where the electron acceptors nitrate, sulfate and iron(III) have been depleted, alkanes are also degraded by methanogenic communities. Zengler et al showed that anaerobic communities degraded hexadecane to methane and carbon dioxide with the most prevalent gas being methane (73). In a similar study, Anderson and Lovley showed that methane and carbon dioxide were produced from radiolabeled hexadecane in oil-contaminated anaerobic sediments. The sediments were shown to be depleted of nitrate, sulfate and iron(III). The molar ratios of the radiolabeled gases (methane and carbon dioxide) were consistent with results shown by Zengler et al (2, 73).

Studies have shown that a wide range of *n*-alkanes are degraded by anaerobic microorganisms. Strain Hxd3 was shown to utilize *n*-alkanes ranging C_{12} to C_{20} (1) while strain TD3, a thermophilic marine microorganism, utilized *n*-alkanes ranging from C_6 to C_{16} with concomitant reduction of sulfate (63). Caldwell et al determined the extent of removal of saturated *n*-alkanes in weathered North Slope crude oil using marine sediments as inocula. Results showed that *n*-alkanes, ranging from C_{15} to C_{34}, were susceptible to biodegradation using sulfate as the predominant electron acceptor (14).

Alicyclic Hydrocarbons

Alicyclic hydrocarbons comprise a significant fraction of organic components in petroleum sources such as gasoline, crude oil and gas condensates. In gasoline and gas condensates, this group of hydrocarbons represented 11 to 12 % (weight/weight) of total hydrocarbons and up to 12% in crude oil depending upon the source (60). Rios-Hernandez et al studied the anaerobic degradation of ethyl cyclopentane (ECP) under sulfate-reducing conditions using an enriched culture from a gas condensate-contaminated aquifer. Their studies showed that ECP was activated by an addition reaction to fumarate as shown for alkane activation. In sediment-free enrichment cultures, the authors demonstrated degradation of ECP with concomitant reduction in sulfate concentration. The amount of expected sulfate utilization was 94% of theoretical assuming complete mineralization of ECP (60).

Townsend et al conducted further studies with gas condensate-contaminated aquifer samples by amending the samples with gasoline. Results showed that the volatile alicyclic hydrocarbons, cyclopentene, methylcyclopentane, cyclohexane, methylcyclopentene and methylcyclohexane, were completely degraded under sulfate-reducing conditions by 100 days but less effectively under methanogenic conditions (68).

ANAEROBIC DEGRADATION OF AROMATIC HYDROCARBONS

BTEX

The BTEX compounds, components of crude oil and other petroleum sources, have higher water solubility than aliphatic, alicyclic and PAHs. Thus, there is a greater risk of exposure due to their dispersion in contaminated groundwater resulting from leaks, spills or releases. The significance of BTEX contamination is further heightened by the fact that benzene is a known carcinogen. The TEX compounds are not carcinogenic but must be kept at minimal concentrations in drinking water and recreational waters due to their toxicity (43).

Benzene

Studies have demonstrated that benzene is susceptible to anaerobic attack. Investigations by Edwards and Grbic-Galic demonstrated that benzene was completely degraded in microcosms containing subsurface sediments using sulfate as the assumed terminal electron acceptor. Results showed that greater than 90% of [^{14}C]-labeled benzene was mineralized to [^{14}C]-labeled carbon dioxide (26). Lovley et al showed that benzene was oxidized to carbon dioxide with sulfate as the terminal electron acceptor. Analysis of radiolabeled carbon dioxide showed that over 92% of [^{14}C]-labeled benzene was recovered as [^{14}C]-labeled carbon dioxide (46). Phelps et al developed a sediment-free stable benzene-degrading culture with sulfate as the terminal electron acceptor. The sediment-free culture had been grown on benzene as the sole carbon and energy source for over 3 years. As individual strains could not be purified from the microbial consortium, the investigators examined 16S ribosomal RNA genes using molecular approaches including cloning, sequencing and PCR fingerprint methodologies. Results showed that 12 genotypes were identified (55).

Benzene has also been degraded using nitrate or iron(III) as the terminal electron acceptor. Burland and Edwards demonstrated that benzene oxidation linked to nitrate reduction occurred in soil-groundwater microcosms. In experiments with [^{14}C]-labeled benzene, results showed that 92 to 95% of the label was recovered in [^{14}C]-labeled carbon dioxide while 5 to 8% was recovered in the nonvolatile fraction, presumably cell mass (13). Coates et al demonstrated benzene oxidation coupled to nitrate or perchlorate reduction with production of [^{14}C]-labeled carbon dioxide using pure strains of *Dechloromonas* sp. Benzene concentrations as high as 160 μM were degraded within 5 days (18). Studies showed that

greater than 86% of the theoretical ratio of benzene oxidation coupled to nitrate reduction was achieved. In a review, Coates summarized potential mechanisms in the anaerobic degradation of benzene (17).

Benzene degradation has also been demonstrated in methanogenic sediments. Weiner and Lovley demonstrated rapid benzene degradation in methanogenic sediments collected from a petroleum-contaminated aquifer. Previous studies had shown that considerable time periods (up to 400 days) were required for cultures to adapt and/or amendments added to stimulate benzene-degrading activity. Due to the length of time, it was questioned whether benzene was actually being degraded *in situ*. [^{14}C]-labeled benzene was degraded to equal amounts of [^{14}C]-labeled methane and [^{14}C]-labeled carbon dioxide without an apparent adaptation period suggesting that benzene was being degraded *in situ*. Nitrate and sulfate were not detected and iron(III) was present in very low levels in groundwater samples suggesting that methane production was the terminal electron-accepting process in this aquifer. Acetate, propionate and phenol were potential intermediates in benzene breakdown as evidenced by isotope trapping experiments (69).

Toluene

Anaerobic degradation of toluene has been extensively studied by a number of investigators with enriched or pure cultures. Pure cultures able to oxidize toluene include those that reduce nitrate (24, 29, 30, 31, 58), sulfate (8, 35, 59) and iron(III) (47, 48). Other cultures include a binary syntrophic culture (51) and an anoxygenic phototrophic microorganism (74).

The metabolic pathway for the anaerobic degradation of toluene has been investigated by a number of research groups. In early studies, benzylsuccinic and benzylfumaric acid was detected in culture extracts of toluene-degrading sulfate-reducing (9) and nitrate-reducing enrichment cultures (28) and were thought to be dead-end metabolites. In studies with the pure denitrifying culture, *Thauera aromatica*, results showed that benzylsuccinate was a true intermediate and was produced by a novel addition reaction to fumarate forming benzylsuccinate that was catalyzed by the enzyme, benzylsuccinate synthase (10). Benzylsuccinate was converted to benzoyl-CoA through a modified β-oxidation pathway with the release of succinate. Benzoyl-CoA is reduced by benzoyl-CoA reductase and further degraded via β-oxidation to acetyl-CoA and carbon dioxide (36). In other studies, toluene activation by benzylsuccinate synthase was demonstrated in toluene-degrading nitrate-reducing and sulfate-reducing cultures (57), an enriched methanogenic culture (7) and in a phototrophic culture (74).

Ethylbenzene

There have been a number of investigations on the anaerobic biodegradation of ethylbenzene under nitrate- and sulfate-reducing conditions (67). In studies with *Azoarcus* sp. strain EB1, Ball et al showed that ethylbenzene was mineralized to carbon dioxide under denitrifying conditions as evidenced by conversion of 69% of [^{14}C-labeled]-ethylbenzene to [^{14}C-labeled]-carbon dioxide, 23% in the nonvolatile fraction (biomass and possible nonvolatile metabolites) and 8% in the volatile fraction (volatile metabolites). Ethylbenzene oxidation was coupled to nitrate reduction and the strain could not utilize ethylbenzene with oxygen as a terminal electron acceptor. Several metabolic intermediates were identified and included 1-phenylethanol and acetophenone. Studies also showed that carbon dioxide was required for growth on acetophenone. With these results, the authors proposed that ethylbenzene was converted to 1-phenylethanol, dehydrogenated to acetophenone, carboxylated to benzoyl acetate, activated to benzoyl acetyl-CoA and thiolytically cleaved to acetyl-CoA and benzoyl CoA (5). In follow up studies, *in vitro* assays were developed for measuring ethylbenzene dehydrogenase and 1-phenylethanol dehydrogenase activities in cell extracts of strain EB1 (39).

Rabus and Widdel isolated strain EbN1, a strain able to mineralize ethylbenzene under denitrifying conditions. In addition to strain EbN1, denitrifying strains PbN1, ToN1 and mXyN1 were isolated from subcultures growing on propylbenzene, toluene and *m*-xylene, respectively. Degradation balance studies showed that strain EbN1 completely oxidized ethylbenzene to carbon dioxide (58). Follow-up experiments confirmed the presence of the key metabolic intermediates, 1-phenylethanol and acetophenone in strain EbN1 as previously shown by Ball et al (5, 57). Since strain EbN1 was also capable of utilizing toluene, additional studies were conducted and showed that the ethylbenzene and toluene metabolic pathways were independent of each other and inducible by respective substrates (16).

Kniemeyer et al conducted studies with marine sediments and isolated an ethylbenzene-degrading microorganism, strain EbS7, under sulfate-reducing conditions. This strain was found to utilize a different metabolic pathway than utilized by denitrifying microorganisms. Investigations showed that the pathway was similar to that used in the anaerobic degradation of *n*-alkanes where a subterminal carbon reacted with fumarate to form (1-phenylethyl)-succinate as evidenced by detection of this metabolite in supernatant extracts as well as 4-phenylpentanoate. From these data, the authors proposed a pathway where ethylbenzene reacts with fumarate to form (1-phenylethyl)-succinate, carbon skeletal rearrangement of the succinyl moiety (as the CoA derivative) and loss of carbon dioxide to yield 4-phenylpentanoyl-CoA. No experimental evidence was presented on further degradation of 4-phenylpentanoyl-CoA (40).

Xylenes

A number of studies have been conducted on the susceptibility of xylenes to anaerobic biodegradation under nitrate-reducing, sulfate-reducing and fermentation (methanogenic) conditions. Krieger et al conducted studies with permeabilized cells of *Azoarcus* sp. Strain T, a denitrifying strain capable of degrading *m*-xylene. The investigators showed that the strain catalyzed the addition of *m*-xylene to fumarate forming (3-methylbenzyl)succinate (3-MeBS) in a metabolic pathway analogous to toluene. 3-MeBS was ultimately converted to 3-methylbenzoyl-CoA and further degraded through central metabolic pathways. It was of interest that permeabilized cells grown on *m*-xylene were also able to metabolize *o*-xylene and *p*-xylene (41). Other investigators have demonstrated transformation or mineralization of *m*-xylene (24, 30, 31, 38, 58), *o*-xylene (28, 30) and *p*-xylene (34) under denitrifying conditions using pure or enrichment cultures.

Harms et al isolated strains oXyS1 and mXyS1 which were able to mineralize *o*-xylene and *m*-xylene, respectively, to carbon dioxide under sulfate-reducing conditions with over 90% of either substrate removed (35). Other investigators demonstrated anaerobic degradation of *p*-xylene under sulfate-reducing conditions (53, 54). Edwards and Grbic-Galic showed that *o*-xylene was degraded by a methanogenic consortium (25).

Polycyclic Aromatic Hydrocarbons (PAHs)

As with BTEX contaminants, PAHs are considered to be an important contaminant in groundwater and sediments. Selected PAHs have been shown to be degraded using nitrate, sulfate, iron(III) as electron acceptors but not under fermentative (methanogenic) conditions (49).

Early investigators showed that naphthalene and acenaphthene were susceptible to anaerobic degradation under nitrate-reducing conditions in soil-water systems (52). Rockne et al reported the isolation of pure cultures capable of degrading naphthalene under denitrifying conditions from an enriched denitrifying naphthalene-degrading consortium (61).

Most investigators have focused their studies on naphthalene degradation under sulfate–reducing conditions. Galushko et al reported that a pure culture (strain NaphS2), able to degrade naphthalene under sulfate-reducing conditions, was isolated from enrichment cultures developed from marine sediments (33).

Meckenstock et al showed that a naphthalene-degrading enrichment culture, developed from contaminated aquifer samples under sulfate-reducing conditions, oxidized naphthalene to carbon dioxide. A number of substrates were tested to determine if they were intermediates in the metabolic pathway. Results showed that the culture was able to oxidize naphthalene, 2-methylnaphthalene, 1- and 2-naphthoic acids, phenylacetic acid, benzoic acid, cyclohexanecarboxylic acid and cyclohex-1-ene-carboxylic acid. Reduced 2-naphthoic acid metabolites were identified and included 1,2,3,4-tetrahydro-2-naphthoic acid, and decahydro-2-naphthoic acid. Other metabolites such as 5,6,7,8-tetrahydro-2-napththoic acid and octahydro-2-naphthoic acid were tentatively identified. Studies also showed that naphthalene was activated by carboxylation as the initial step in the metabolic pathway (50).

In a follow-up study, Annweiler et al reported that 2-methylnaphthalene was degraded under sulfate-reducing conditions using an enrichment culture developed from freshwater sediment. Metabolite analysis of culture extracts showed that 2-methylnaphthalene was activated by fumarate addition to the methyl group in a similar reaction shown with the degradation of toluene (4). Other metabolites identified included 2-naphthoic acid and the reduced 2-naphthoic acid compounds, 5,6,7,8-tetrahydro-2-naphthoic acid, octahydro-2-naphthoic acid and decahydro-2-naphthoic acid. A metabolic pathway for the anaerobic degradation of 2-methylnaphthalene was proposed. Similar results were reported by Galushko et al using a 2-methylnaphthalene-degrading enrichment culture of mesophilic freshwater bacteria under sulfate-reducing conditions (32).

In further studies on metabolite analysis with enrichment culture N47, Annweiler et al identified metabolites in cultures growing on naphthalene, 2-methylnaphthalene and tetralin (1,2,3,4-tetrahydro-2-naphthalene). Gas chromatography-mass spectrometry (GC-MS) studies showed that naphthalene and 2-methylnaphthalene were activated by direct carboxylation and addition to fumarate, respectively, with further degradation leading to 2-naphthoic acid. Evaluation of reduced 2-naphthoic acid metabolites showed that tetrahydro-, octahydro- and decahydro-2-naphthoic acid were found in supernatants of cultures grown with naphthalene, 2-methylnaphthalene and tetralin. Two common ring cleavage compounds were also identified. A metabolic pathway for the anaerobic degradation of bicyclic aromatic hydrocarbons with 2-naphthoic acid as the central intermediate was proposed. (3).

The activation of naphthalene has not been completely clarified. Studies by Zhang and Young showed that carboxylation is the initial key reaction in the anaerobic degradation of naphthalene and phenanthrene. These studies were carried out using an enrichment culture developed from sediments collected from the Arthur Kill in the New York/New Jersey Harbor Estuary. Results showed that [^{14}C]-labeled naphthalene and phenanthrene were mineralized to [^{14}C]-labeled carbon dioxide. 2-naphthonoate was shown to be a major metabolite in enrichment cultures when halogenated analogs were added. When the enrichment culture was provided with naphthalene or phenanthrene in the presence of [^{13}C]-labeled bicarbonate, the labeled bicarbonate was incorporated into either substrate with the production of 2-naphthoate and phenanthrenecarboxylic acid, respectively. The authors concluded that carboxylation was a key initial step for activating naphthalene and phenanthrene by anaerobic cultures (75). Similar results were found by Davidova et al in experiments evaluating the anerobic degradation of phenanthrene in marine sediments collected from the San Diego Bay. Studies showed that phenanthrene degradation was stoichiometrically in agreement with the theoretical amount of sulfate reduced. Mineralization of [^{14}C]-labeled phenanthrene to the expected amount of recovered [^{14}C]-labeled carbon dioxide was also confirmed. These authors demonstrated incorporation of [^{13}C]-labeled bicarbonate into the carboxyl

group of phenanthrene-2-carboxylic acid and suggested that direct carboxylation of phenanthrene was a likely key reaction (23).

In contrast, Safinowski and Meckenstock reported that methylation of naphthalene was the initial step for degrading naphthalene by enrichment culture N47. In these studies, the key metabolites and enzymes found in 2-methylnaphthalene degradation were identified in samples collected from cultures actively degrading naphthalene. They proposed that the methyl group was generated from bicarbonate by a reversed carbon monoxide (CO) dehydrogenase pathway. This enzyme was detected in cell-free extracts of N47. They proposed that naphthalene was methylated to 2-methylnaphthalene and further metabolized using the pathway for 2-methylnaphthalene degradation as previously described (64).

Coates et al demonstrated that [^{14}C]-labeled naphthalene and phenanthrene were oxidized to [^{14}C]-labeled carbon dioxide in San Diego Bay sediments heavily contaminated with PAHs. PAH oxidation occurred without a lag period and only in the presence of sulfate as an electron acceptor. When molybdate, an inhibitor of sulfate reduction, was added, oxidation of [^{14}C]-labeled naphthalene or labeled phenanthrene ceased which further confirmed that PAH oxidation was linked to sulfate reduction (21).

Coates et al added iron(III) oxide to sediments in an attempt to stimulate *in situ* hydrocarbon degradation in anaerobic petroleum-contaminated San Diego Bay sediments where sulfate was the principle terminal electron accepting process. Previous studies showed the iron(III) reducing bacteria can oxidize a variety of electron donors more efficiently than sulfate-reducing bacteria. Addition of iron(III) oxide to harbor sediments would provide a terminal electron acceptor on a long term basis and theoretically provide an efficient means of contaminant removal by anaerobic degradation. [^{14}C]-labeled benzene, naphthalene and toluene were added to anaerobic sediments and [^{14}C]-labeled carbon dioxide monitored. Results showed that benzene and toluene were actively converted to [^{14}C]-labeled carbon dioxide but the rate of oxidation was the same whether iron(III) oxide was added or not. These data indicated that the addition of iron(III) oxide did not stimulate hydrocarbon oxidation and did not switch the terminal electron accepting process from sulfate to iron(III). Radiolabeled naphthalene was added to sediments and converted to [^{14}C]-labeled carbon dioxide after a lag period of 70 days. However, when experiments were conducted with sediments, air-oxidized to increase iron(III) concentrations and to selectively remove sulfate-reducing microbial populations, naphthalene was not oxidized. Enumeration of iron(III)- and sulfate-reducing microbial populations by most probable number (MPN) analysis showed that iron(III)-reducing bacteria were 3 orders of magnitude lower that sulfate-reducing bacteria in harbor sediments suggesting that iron(III)-reducing bacteria could not compete with sulfate reducers due to low populations. The authors concluded that addition of iron(III) oxides would not likely enhance the degradation of organic contaminants in San Diego Bay sediments (20).

In a follow-up study, Coates et al evaluated the susceptibility of a number of other PAHs to anaerobic degradation besides naphthalene and phenanthrene. Results showed that naphthalene, methylnaphthalene, fluorene, phenanthrene and fluoranthene were susceptible to anaerobic degradation as evidenced by the production of [^{14}C]-labeled carbon dioxide from the [^{14}C]-labeled PAH source. Minor amounts of [^{14}C]-labeled methane were detected in these sediments. In contrast, degradation of pyrene or benzo(*a*)pyrene did not occur in the 37-day study as [^{14}C]-labeled carbon dioxide and [^{14}C]-labeled methane were not detected. The investigators also showed that anaerobic degradation of alkanes occurred in these sediments. Diesel marine fuel and JP-5 jet fuel were added to sediments and both fuels were substantially degraded after 80 days. Results showed that susceptible alkanes ranged from C_{11} to C_{24} in these complex fuel mixtures and the authors concluded that all of the major alkane components of diesel marine and JP-5 jet fuels could be degraded under sulfate-reducing conditions (19).

Rothermich et al evaluated the *in situ* anaerobic degradation of PAHs in anoxic microcosms of coal tar-contaminated Boston Harbor sediments. Results showed that two-ringed (naphthalene, 1-

methylnaphthalene, 2-methylnaphthalene), three-ringed (acenaphthene, fluorene, phenanthrene, anthracene) and four- and five-ringed (fluoranthene, pyrene, benzo(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene) PAHs showed measurable reduction in concentration over an 11-month study. The authors concluded that anaerobic microorganisms can significantly impact PAH concentrations in petroleum hydrocarbon-contaminated, anaerobic and sulfate-reducing harbor sediments *in situ* (62).

SUMMARY

A review of the literature on the anaerobic degradation of petroleum hydrocarbons was conducted. This summary has attempted to capture the current state of research efforts on the degradation of saturated, aromatic and PAH hydrocarbons. The review focused on the array of petroleum hydrocarbons that are susceptible to anaerobic degradation and provided a physiological basis for degradation. Research on the anaerobic degradation of hydrocarbons is relatively new with most of the papers discussed in this review published in the last 20 years. Since the papers were primarily summaries of laboratory studies, it is not clear whether laboratory results can be translated into an engineering design strategy for successful site cleanup by anaerobic bioremediation technology.

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